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(54) Title: USE OF XYLANASE IN BAKING

(57) Abstract

A method of improving properties of a dough and/or a baked product made from dough by adding an enzyme preparation to the dough and/or to any ingredient of the dough and/or to any mixture of the dough ingredients, in which method the enzyme preparation comprises a xylanase obtainable from a strain of the fungal species *A. aculeatus*. The xylanase may be present in a bread or dough improving composition.

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Use of Xylanase in Baking

FIELD OF THE INVENTION

The present invention relates to a method of improving properties of a dough and/or of a baked product made from dough by use of a xylanase preparation. Furthermore, the invention relates to a dough and baked product produced by the method, as well as to a dough conditioner, a bread-improving composition and a pre-mix comprising xylanase for the preparation of dough.

BACKGROUND OF THE INVENTION

In the bread-making process it is known to add bread-improving additives and/or dough conditioners to the bread dough, the action of which, inter alia, results in improved texture, volume, flavour and freshness of the bread as well as improved machinability of the dough.

In recent years xylanases have gained considerable importance for use in the preparation of bread and baked products, in particular so as to increase the volume and anti-staling of bread and other baked products.

For instance, EP 396 162, EP 493 850 and EP 487 122 relate to bread improvers, deep-frozen dough and fat-free pastry mix, respectively, comprising xylanase optionally in combination with other enzymes. WO 91/18977 discloses a method of preparing a pentosanase-containing preparation having increased baking activity.

One drawback associated with the use of xylanases is, however, that dough prepared with xylanase tends to become too sticky and thus difficult to handle. Accordingly, when xylanases are used for baking it is necessary to avoid that the dough becomes too sticky, either by reducing the amount of xylanase used (and thereby the increase of the volume to be obtained), or by using

other measures (e.g. other enzyme activities) to reduce the dough stickiness caused by the xylanase.

EP 321 811 relates to the use of glucose oxidase in combination with a cellulase and/or hemicellulase (such as xylanase) and optionally sulfhydryl oxidase.

PCT/DK93/00274 discloses the use of lipase in the preparation of dough and baked products. It is mentioned that xylanase may be used in combination with the lipase. However, the xylanase is not further specified and the only use of said type of xylanase is in combination with lipase.

DISCLOSURE OF THE INVENTION

It has now surprisingly been found that by use of a particular type of xylanase it is possible to obtain an increase in volume of bread and other baked products which is substantially larger than that obtained with a known xylanase, without at the same time obtaining a dough which is too sticky.

Accordingly, in a first aspect the present invention relates to a method of improving properties of a dough and/or a baked product made from dough by adding an enzyme preparation to the dough and/or to any ingredient of the dough and/or to any mixture of the dough ingredients, in which method the enzyme preparation comprises a xylanase obtainable from a strain of the fungal species *Aspergillus aculeatus*.

In the present context, the term "obtainable" is intended to indicate that the xylanase may be obtained from a strain of the fungal species *A. aculeatus*, either by being produced by and recovered from such strain, or by being encoded by a DNA sequence isolated from such strain and produced in a host organism transformed with said DNA sequence.

Alternatively, the term is intended to cover a xylanase obtained from other sources, in particular other microbial sources, which xylanase is homologous with a *A. aculeatus* xylanase. In particular, the term is intended to cover any of the xylanases described in WO 94/21785.

As far as the present inventor is aware no prior disclosure of the use of an *A. aculeatus* xylanase for baking exists. WO 93/00274 (which was not published at the priority date of the present application) mentions that lipase may be used in combination with an *A. aculeatus* xylanase for baking, but not that specific *A. aculeatus* xylanases, when used without a lipase, have highly interesting properties.

The term "improved properties" as used about the effect obtained on dough and/or baked products made from dough according to the present invention is intended to be understood broadly, i.e. to include any property which may be improved by the action of xylanase (in comparison with properties obtained when no xylanase has been added).

As indicated above, a particular advantage associated with the method of the invention is that an increased volume and improved softness of the baked product may be obtained. Also, an improved crumb softness upon storage is obtained.

In further aspects the present invention relates to a dough or a baked product which has been prepared by a method of the invention, to a dough conditioner, a bread-improving composition and a pre-mix for dough and/or baked products comprising the xylanase defined above.

In a final aspect, the invention relates to the use of a xylanase as defined above for the preparation of dough and/or baked products.

DETAILED DESCRIPTION OF THE INVENTION

The xylanase to be used in the present invention is preferably one, which is capable of increasing the volume of a loaf (prepared as described in the examples below) with at least 20% from a dough having a nice soft appearance - i.e. a dough which is not too sticky to be handled in an efficient manner. The stickiness is normally evaluated visually, e.g. as described in the Materials and Methods section herein, and an acceptable stickiness is normally considered to be 3-3.5. More preferably, an increase in volume of at least 25%, such as at least 30% may be obtained in accordance with the invention.

Preferred examples of xylanases to be used in the present invention include any of the xylanases described in WO 94/21785 (incorporated herein by reference), in particular a xylanase derived from a strain of *Aspergillus*, such as *A. aculeatus*, e.g. the strain CBS 101.43, or a xylanase homologous thereto (as defined in WO 94/21785), e.g. derived from a strain of the fungal genus *Humicola*, *Trichoderma*, *Fusarium* or *Scytallidium*.

In particular the xylanase to be used in the present invention is *A. aculeatus* xylanase I or *A. aculeatus* xylanase II (as defined in WO 94/21785). These xylanases are encoded by the DNA sequence shown in SEQ ID No. 1 and SEQ ID No. 2 of WO 94/21785, respectively, and may be produced as described in WO 94/21785 by fermentation of a suitable strain transformed with (or inherently comprising) the relevant DNA sequence and capable of expressing the enzyme and subsequent recovery of the enzyme from the fermentation broth.

In the present context the terms "xylanase I" and "xylanase II" are intended to denote xylanases encoded by the DNA sequence shown in SEQ ID Nos. 1 and 2 of WO 94/21785 as well as homologues of these xylanases (as defined in WO 94/21785). For instance, the homologue may be a xylanase dffering in one or more amino acid residues as compared to the xylanase encoded by SEQ ID No 1 or 2 of WO 94/21785, but which has retained

essentially the same xylanolytic activity as the xylanase encoded by SEQ ID No 1 or 2.

It is well known when working with pentosanases or xylanases in baking that an enzyme activity analysis based on soluble or insoluble pentosans or arabinoxylans substrates can not be correlated to the baking performances of different enzyme preparations from different microorganisms (Joan Qi Si et al., Presentation at the AACC Annual Congress, 1993 Miami Beach. Abstract No. 308).

Therefore, the optimum dosage of the xylanase to be used must be determined experimentally. The optimum dosage can be defined as the dosage that provides the maximum specific volume increase without getting a too sticky dough. The dosage can be standardized as g enzyme per kg flour, mg protein per kg flour or a given analysis activity per kg flour. In the present invention FXU per kg flour has been chosen in order to have a standard. However, it must be understood that the dosage of *A. aculeatus* xylanases (when expressed in FXU per kg of flour) cannot be compared directly with the dosage of other xylanases (expressed in the same activity) as the activity does not fully correlate with baking performance. Accordingly, when comparing other xylanases with the xylanases to be used in the present invention, it is essential that the baking performance (in terms of optimal volume and low dough stickiness) is chosen as the parameter to compare, cf also the examples hereinafter.

In order to obtain the maximum possible volume effect by the method of the present invention the xylanase to be used is preferably added in an amount which results in a dough stickiness of at the most 3.0 or more preferably at the most 3.5 as defined herein. For the *A. aculeatus* xylanases exemplified in the present application this dosage corresponds to 5-5000 FXU/kg of flour, such as 20-2000 FXU/kg of flour. A preferred dosage is in the range of 50-1500 FXU/kg of flour such as 250-1000 FXU/kg of flour.

The xylanase activity FXU (Farbe-Xylanase-Units) may be determined by the procedure given in the Materials and Methods section below.

While the xylanase defined herein may be used as the only or substantially only enzymatic activity, the properties of dough and/or baked products may be further improved when the xylanase is used in combination with a further enzymatic activity.

Such further enzymatic acitivity may either be one present in the xylanase preparation recovered from the organism producing it, or may, more preferably, be added to the xylanase preparation.

Examples of other enzymes are a cellulase, a hemicellulase, a glucose oxidase (useful for strengthening the dough), e.g. a fungal glucose oxidase such as Novozym 358® (a *A. niger* glucose oxidase), a protease (useful for gluten weakening in particular when using hard wheat flour), e.g. Neutrase®, a peroxidase (useful for improving dough consistency), a lipase (useful for reducing the dough stickiness caused by xylanase and an overall volume increase), a peptidase, a maltogenase, and/or an amylase, such as an amyloglucosidase (e.g. AMG® (an *A. niger* amyloglucosidase) and an α-amylase (useful for providing sugars fermentable by yeast). The other enzymes are preferably of microbial origin and may be obtained by conventional techniques used in the art as mentioned above.

In particular, the xylanase is used in combination with a lipase and optionally other enzymes.

At present, the most preferred combination of enzymes to be used in the present invention comprises *A. aculeatus* xylanase I and/or II as described in WO 94/21785 in admixture with a lipase, an amylase and/or an oxidase (e.g. a glucose oxidase or a peroxidase).

In another embodiment of the method of the invention xylanase is advantageously used in combination with an amylase such as an α-amylase or an amyloglucosidase. The amylase is preferably of microbial origin, e.g. derived from a bacterium or fungus, such as a strain of *Aspergillus*, in particular of *A. niger* or *A. oryzae*, or a strain of *Bacillus*. Commercially available α-amylases suited for the present purpose are Fungamyl® (an *A. oryzae* α-amylase), Novamyl® (a *B. stearothermophilus* maltogenic α-amylase, cf. EP 120 693), and BAN® (a *B. amyloliquefaciens* α-amylase) all available from Novo Nordisk A/S. Further useful amylase products include Grindamyl (A 1000 and A 5000) from Grindsted Products (Danisco, Denmark) and Amylase H and Amylase P from Gist-Brocades, The Netherlands.

The other enzyme activities may be dosed in accordance with established baking practice. In this respect, a preferred dosage of lipase is in the range of 10-100,000 LU/kg of flour, such as 25-50,000 LU/kg of flour, e.g. in the range of 50-5000 LU/kg of flour. A preferred dosage of amylase is 5-500 FAU/kg of flour. FAU and LU are further defined in the Materials and Methods section below.

While the enzymes to be used in the present invention may be of any origin, including animals and plants, microbial enzymes are preferred inter alia, because such enzymes may normally be prepared in large amounts by fermentation of suitable micro-organisms. Furthermore, microbial enzymes may generally be obtained in a higher purity than other types of enzymes resulting in a lower amount of undesirable side-activities, such as undesirable non-xylanolytic activities.

The microbial enzymes to be used in the methods of the invention may be of bacterial, yeast or fungal origin.

The enzymes may be obtained from the microorganism in question by use of any suitable technique. For instance, a xylanase preparation may be obtained by fermentation of a microorganism and subsequent isolation by a method known in the art, but more

preferably by use of recombinant DNA techniques as known in the art. Such method normally comprises cultivation of a host cell transformed with a recombinant DNA vector capable of expressing and carrying a DNA sequence encoding the xylanase in question, in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture.

The DNA sequence encoding the xylanase to be used may be of any origin, e.g. a cDNA sequence, a genomic sequence, a synthetic sequence or any combination thereof. WO 94/21785 describes suitable methods for preparing xylanases to be used in the present invention.

As indicated above, the enzyme preparation to be used in a method of the invention may comprise one or more enzyme activities in addition to the xylanase, e.g. activities which are produced by or recovered from the xylanase source in question. Thus, any other components present in the enzyme preparation may be of a different or the same origin as the xylanase. Alternatively, one or more additional enzyme activities may be added separately from the enzyme preparation comprising the xylanase.

Suitable examples of a microbial lipase to be used for the present purpose include a lipase derived from a strain of *Humicola* spp., *Rhizomucor* spp., *Candida* spp., *Aspergillus* spp., *Rhizopus* spp. or *Pseudomonas* spp., especially from a strain of *H. lanuginosa*, *Rh. miehei*, *C. antarctica*, *Aspergillus niger* or *Pseudomonas cepacia*. Specific examples of such lipases are lipase A and lipase B of *C. antarctica*, e.g. described in WO 88/02775, the *Rh. meihei* lipase described by Boel et al., 1988, Huge-Jensen et al., 1989 and in EP 238 023, the *H. lanuginosa* lipase described in EP 305 216, and the *P. cepacia* lipase described in EP 214 761 and WO 89/01032.

Besides the above mentioned additional enzyme activities a microbially produced xylanase preparation may contain varying

minor amounts of other enzymatic activities inherently produced by the producer organism in question.

The enzyme preparation to be used in the present invention may advantageously be used in combination with other dough conditioners or bread improvers.

The enzyme preparation to be used in the method of the invention may be in any form suited for the use in question, e.g. in the form of a dry powder or granulate, in particular a non-dusting granulate, a liquid, in particular a stabilized liquid, or a protected enzyme. Granulates may be produced, e.g. as disclosed in US 4,106,991 and US 4,661,452, and may optionally be coated by methods known in the art. Liquid enzyme preparations may, for instance, be stabilized by adding nutritionally acceptable stabilizers such as a sugar, a sugar alcohol or another polyol, lactic acid or another organic acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216. The enzyme preparation of the invention may also comprise a preservative.

Normally, for inclusion in pre-mixes or flour it is advantageous that the enzyme preparation is in the form of a dry product, e.g. a non-dusting granulate, whereas for inclusion together with a liquid it is advantageously in a liquid form.

The enzyme preparation may be added as such to the mixture from which the dough is made, or may, alternatively, be added as a constituent of a dough conditioner and/or a bread-improving composition. The dough conditioner and/or the bread-improving composition may be any conventionally used composition, e.g. comprising one or more of the following constituents:

A milk powder (providing crust colour), an emulsifier (e.g. mono- or diglycerides, e.g. DATEM and SSL, diacetyl tartaric acid esters of mono- or diglycerides, sugar esters of fatty acids, polyglycerol esters of fatty acids, lactic acid esters of monoglycerides, acetic acid or citric acid esters of

monoglycerides, polyoxyethylene stearates, phospholipids and lecithin), granulated fat (for dough softening and consistency of bread), and oxidant (added to strengthen the gluten structure; e.g. ascorbic acid, potassium bromate, potassium iodate or ammonium persulfate), an amino acid (e.g. cysteine), a sugar, salt (e.g. sodium chloride, calcium acetate, sodium sulfate or calcium sulfate serving to make the dough firmer) and gluten (to improve the gas retention power of weak flours).

Typically, the dough conditioner and/or the bread-improving composition is added in an amount corresponding to about 0.1-5%, such as 0.5-3% of the added flour.

The method of the present invention is contemplated to be useful in increasing the volume and anti-staling properties of a baked product without, however, severely imparting the machinability of the dough. Of course, the improved machinability is particularly important for dough types to be processed industrially, an example of which is dough types which are to be extruded (e.g. for the preparation of bisquits or other crisp products).

As it is indicated above, the term "baked product" is intended to include any product prepared from dough. The baked product may be yeast-leavened or chemically leavened and may be of a soft or a crisp character. Examples of baked products, whether of a white, light or dark type, which may advantageously be produced by the present invention are bread, typically in the form of loaves or rolls, French baguette-type bread, pita bread, tacos, cakes, pan-cakes, waffles, bisquites, crisp bread and the like.

The dough and/or baked product prepared by the method of the invention are normally based on wheat meal (including whole meal) or flour, optionally in combination with other types of meal or flour such as corn flour, rye meal, rye flour, oat flour or meal, soy flour, sorghum meal or flour, or potato meal or flour. However, it is contemplated that the method of the

present invention will function equally well in the preparation of dough and baked products primarily based on other meals or flours, such as corn meal or flour, rye meal or flour, or any other types such as the types of meal or flour mentioned above. The dough may be substantially free from added fat or may contain even considerable amounts of fat (e.g. butter, margarine, shortening, oil, or the like).

As mentioned above the xylanase preparation is added to any mixture of dough ingredients, to the dough, or to any of the ingredients to be included in the dough, in other words the xylanase preparation may be added in any step of the dough preparation and may be added in one, two or more steps, where appropriate. However, the xylanase should not be added together with any strong chemical or under conditions where the enzyme is inactivated.

The handling of the dough and/or baking is performed in any suitable manner for the dough and/or baked product in question, typically including the steps of kneading of the dough, subjecting the dough to one or more proofing treatments, and baking the product under suitable conditions, i.e. at a suitable temperature and for a sufficient periode of time. For instance, the dough may be prepared by using a normal straight dough process, a sour dough process, an overnight dough method, a low-temperature and long-time fermentation method, a frozen dough method, the Chorleywood Bread process, and the Sponge and Dough process.

In a further aspect the present invention relates to a dough or a baked product prepared by the method of the present invention. The dough and the baked product of the invention has improved qualities as defined above as compared with products which has not been prepared according to the invention. The baked product and the dough of the invention may be of any of the types discussed above, and it is preferred that the dough is fresh or frozen.

The dough conditioner and/or a bread-improving composition of the invention comprising a xylanase optinally in admixture with other enzymes as defined herein may be prepared on the basis of conventional dough conditioners and/or bread-improving compositions known in the art using procedures known in the art. Specific examples of suitable constituents for dough conditioners and/or bread-improving compositions are listed above.

The pre-mix of the invention may be prepared by techniques known in the art on the basis of pre-mix constituents known in the art such as flour, meal, dough-conditioners, bread-improving additives and the like.

The present invention is further illustrated in the following example which is not considered, in any manner, to limit the scope of the invention as defined herein.

MATERIALS AND METHODS

Enzymes

Lipase A: The *Humicola lanuginosa* lipase described in EP 305 216 and produced by recombinant DNA techniques in *Aspergillus oryzae* as described in EP 305 216. The lipase has a specific activity of 4,452,000 LU/g and a FAU/g of less than 0.6.

Xylanase A: A xylanase produced by the *Humicola insolens* strain DSM 1800 available from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH and further described in EP 507 723.

Fungamyl: A commercial fungal α-amylase preparation available from Novo Nordisk A/S, Denmark.

Pentopan: A commercial xylanase preparation available from Novo Nordisk A/S, Denmark.

LU/g (Lipase Units/g), FAU/g (Fungal alpha-Amylase Units/g) and FXU (xylanase units/g) were determined by the following assays:

LU - Lipase Units

Lipase activity was assayed using glycerine tributyrat as a substrate and gum-arabic as an emulsifier. 1 LU (Lipase Unit) is the amount of enzyme which liberates 1 μmol titratable butyric acid per minute at 30°C, pH 7.0. The lipase activity was assayed by pH-stat using Radiometer titrator VIT90, Radiometer, Copenhagen. Further details of the assay are given in Novo Analytical Method AF 95/5, available on request.

FAU - Fungal alpha-Amylase Units

1 FA-unit (FAU) is the amount of enzyme which at 37°C and pH 4.7 breaks down 5260 mg of solid starch per hour. Further details of the assay are given in Novo Analytical Method AF 9.1/3, available on request.

FXU - xylanase activity

The endo-xylanase activity is determined by an assay, in which the xylanase sample is incubated with a remazol-xylan substrate (4-O-methyl-D-glucurono-D-xylan dyed with Remazol Brilliant Blue R, Fluka), pH 6.0. The incubation is performed at 50°C for 30 min. The background of non-degraded dyed substrate is precipitated by ethanol. The remaining blue colour in the supernatant is determined spectrophotometrically at 585 nm and is proportional to the endoxylanase activity.

The endoxylanase activity of the sample is determined relatively to an enzyme standard.

The assay is further described in the publication AF 293.6/1-GB, available upon request from Novo Nordisk A/S, Denmark.

Preparation of bread

White bread were prepared from the following basic recipe:

Basic recipe

Flour(Manitoba)	100 %
Salt	1.5 %
Yeast (fresh)	5.0 %
sugar	1.5 %
Water	58 %

The wheat flour was of the type termed "Manitoba" supplied by "Valsemøllerne", Denmark, October 1993.

Procedure

1. Dough mixing (Spiral mixer)
   2 min. at 700 RPM
   7 min. at 1400 RPM
the mixing time was determined and adjusted by a skilled baker so as to obtain an optimum dough consistence under the testing conditions used.

2. 1st proof: 30°C - 80% RH, 16 min.

3. Scaling and shaping;

4. Final proof: 32°C - 80% RH, 35 min.;

5. Baking: 225°C, 20 min. for rolls and 30 min for loaf.

Evaluation of dough and baked products

Properties of the dough and baked products were determined as follows:

Roll specific volume: the volume of 20 rolls are measured using the traditional rape seed method. The specific volume is calculated as volume ml per g bread. The specific volume of the control (without enzyme) is defined as 100. The relative specific volume index is calculated as:

$$\text{Specific vol. index} = \frac{\text{specific volume of 20 rolls}}{\text{specific volume of 20 control rolls}} \cdot 100$$

Loaf specific volume: the mean value of 4 loaves volume are measured using the same methods as described above.

The dough stickiness and crumb structure are evaluated visually according to the following scale:

Dough stickiness:	almost liquid	1
	too sticky	2
	slightly sticky	3
	nice soft	3.5
	normal	4
	dry	5
Crumb structure:	very poor	1
	poor	2
	non-uniform	3
	uniform/good	4
	very good	5

The softness of bread crumb is measured by a SMS-Texture Analyzer. A plunger with a diameter of 20 mm is pressed on the middle of a 20 mm thick slice of bread, The force needed for the plunger to depress the crumb 5 mm with a speed of 2.0 mm/s is recorded and it is expressed as the crumb firmness. The lower the value, the softer is the crumb. Four slices of each bread are measured and the mean value is used.

EXAMPLES

EXAMPLE 1

The enzyme used was xylanase I, a recombinant *A. aculeatus* xylanase produced in *A. oryzae* as described in Examples 2 and 3 of WO 94/21785. The effect of this xylanase was compared to xylanase A from *H. insolens* and a commercial available pentosanase, Pentopan. The enzymes were added either directly into the baking ingredients mix or it was dispersed in water before being added to the mix. All tests were carried out in at least duplicate and the average results were used. The results obtained are shown in Table 1.

It is apparent from Table 1 that the addition of xylanase I increases the volume of rolls or/and loaves significantly and the effect is larger than that obtained by the prior art xylanase A and Pentopan. At the optimum dosage, i.e. the dosage that gives the maximum specific volume increase without getting a too sticky dough, of the known pentosanase (Pentopan) and xylanase (xylanase A) the max. volume increase is about 10-16%. At the optimum dosage of Xylanase I (about 350-750 FXU per kg flour) a volume increasing of 29-41% can be achieved without causing a too sticky dough. With a longer proofing time at 80%RH and 32°C an even higher volume increase can be achieved. Furthermore, the crumb structure and crumb softness upon storage are also improved.

Table 1

FXU/kg flour		25	50	100	122	200	350	427	500	750	1000	0
Xylanase I	Dough stickiness	4	4	3.5		3.5	3.5		3.5	3	2.5	4
Xylanase I	SP Volume index/-Rolls	103	109	111		119	121		129	141	133	100
Xylanase I	SP Volume index/-/Loaves*	101	102	105		110	110		114	122	121	100
Xylanase I	SP Volume index/-Loaves**						134					
	Crumb structure		3	3.5		3.5	3.5		4			3
	Crumb firmness		0.278	0.282		0.206	0.258		0.169	0.173	0.189	0.399
	Day 4		0.434	0.440		0.345	0.346		0.357	0.320	0.348	0.509
	Day 7		0.589	0.551		0.451	0.561		0.376	0.383	0.380	0.658
Xylanase A	Dough stickiness		3.5	3								
	SP Volume index/-Rolls		108	116								
Pentopan	Dough stickiness				3.5			3				
	SP Volume index/-Rolls				106			111				

* low fermentation time: 35 min.
** longer fermentation time: 50 min.

EXAMPLE 2

In the same manner as described in Example 1, baking trials with xylanase II, a recombinant *A. aculeatus* xylanase produced as described in WO 94/21785, xylanase A and Pentopan were performed. The results obtained are shown in Table 2.

It is apparent from Table 2 that the use of xylanase II increases the volume of rolls or/and loaves significantly and the effect is larger than the prior art xylanase and pentosanase. At the optimum dosage of Xylanase II (i.e. about 200 FXU per kg flour) a volume increasing of 24% is achieved without causing a too sticky dough. Furthermore, the crumb structure and crumb softness upon storage are also improved.

Table 2

FXU/kg flour		25	50	100	122	200	350	427	500	0
	Dough stickiness	4	4	3.5		3-3.5	2.5-3		2.5	
Xylanase II	SP Volume index/Rolls	111	113	123		124	126		134	
	SP Volume index/loaves	103	107	109		113	117		117	
	Crumb structure	3	3.5	4		4	4.5		4.5	3
	Crumb firmness day 1		0.227	0.193		0.209	0.176		0.215	0.399
	Day 4		0.362	0.359		0.358	0.289		0.294	0.509
	Day 7		0.435	0.390		0.433	0.398		0.386	0.658
Xylanase A	Dough stickiness		3.5	3						
	SP Volume index/Rolls		108	116						
Pentopan	Dough stickiness				3.5			3		
	SP Volume index/Rolls				106			111		

* low fermentation time: 35 min.

EXAMPLE 3

Baking tests were carried out by use of xylanase I or xylanase II in combination with Fungamyl, a fungal alpha-amylase, using the above described procedure. The results shown in the below table indicate that combination of xylanase with an alpha-amylase results in further improved volume increasing, improved crumb structure and softer crumb than when using either enzyme alone.

Table 3

Combination with alpha-amylase

FXU/kg flour		50	200	50	200	0
FAU/kg flour		25	25			
Xylanase I	SP Volume index/Rolls	118	123	109	111	100
	Crumb structure	3.5	4	3	3.5	3
	Crumb firmness Day 1		0.141	0.278		0.399
	Day 4		0.242	0.434		0.509
	Day 7		0.336	0.589		0.658
Xylanase II	SP Volume index/Rolls	122	128	113	124	100
	Crumb structure	3.5	4	3.5	4	
	Crumb firmness Day 1	0.131	0.110	0.227		
	Day 4	0.221	0.204	0.362		
	Day 7	0.300	0.273	0.435		

EXAMPLE 4

Baking tests were carried out by use of xylanase I or xylanase II in combination with lipase A using the above described procedure. The results shown in the below table indicate that combination of xylanase with lipase resulting in further increasing of volume, improved crumb structure and softer crumb than when using either enzyme alone. Furthermore, when xylanase is combined with lipase, the lipase provide a dough as dry as the control. When higher amounts of xylanase are used together with lipase, a dry, soft and more elastic dough can be obtained at the same time with a significantly large volume increase and improved crumb structure.

Table 4

Combination with lipase

	FXU/kg flour	50	100	50	100		50	200	200	0
Lipase A	LU/kg flour	250	250	500	500	500				0
	Dough stickiness	4	4	4	4	4.5	4	3 - 3.5	3.5	4
Xylanase I	SP Volume index/Rolls	132	128	126		118	109		119	100
Xylanase II	SP Volume index/Rolls	131		126	135			124		100
	Crumb structure	4	4.5	4.5	4.5	4	3	4	3.5	3

CLAIMS

1. A method of improving properties of a dough and/or a baked product made from dough by adding an enzyme preparation to the dough and/or to any ingredient of the dough and/or to any mixture of the dough ingredients, in which method the enzyme preparation comprises a xylanase obtainable from a strain of the fungal species *A. aculeatus*.

2. The method according to claim 1, in which the xylanase is obtainable from *A. aculeatus* strain CBS 101.43.

3. The method according to claim 1 or 2, in which the xylanase is *A. aculeatus* xylanase I or II.

4. The method according to any of claims 1 - 3, in which the xylanase is added in an amount which results in a dough stickiness of at the most 3.0 as defined herein.

5. The method according to any of claims 1-4, in which enzyme preparation comprises a further enzymatic activity.

6. The method according to claim 5, in which the enzyme preparation comprises an amylase, maltogenase, lipase, cellulase, hemicellulase, pentosanase, peroxidase, glucose oxidase, laccase, protease and/or peroxidase activity.

7. The method according to claim 6, in which the enzyme preparation comprises *A. aculeatus* xylanase I and II in admixture with a lipase, an amylase and/or an oxidase.

8. The method according to any of claims 1-7, in which the enzyme preparation is of microbial origin.

9. A dough or a baked product prepared by the method according to any of claims 1-8.

10. A dough conditioner or bread-improving composition comprising a xylanase obtainable from a strain of the fungal species *A. aculeatus*.

11. The dough conditioner or bread-improving composition according to claim 10, which further comprises a lipase, an amylase and/or an oxidase.

12. A pre-mix for dough and/or baked products which comprises a xylanase obtainable from a strain of *A. aculeatus*, optionally further comprising a lipase, an amylase and/or an oxidase.

13. Use of xylanase obtainable from a strain of the fungal species *A. aculeatus* for improving properties of a dough and/or a baked product made from dough.

14. The use according to claim 16, in which the xylanase is xylanase I or II.

15. The use according to claim 14, in which the xylanase is added to the dough or any ingredient of the dough in an amount resulting in a dough stickiness of the most 3.0 as defined herein.

16. The use according to any of claims 13-15, in which the xylanase is used in combination with a lipase, an amylase and/or an oxidase.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00094

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A21D 8/04
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A21D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALINDEX: FOODSCI, WPI, CLAIMS/US PATENTS, JAPIO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, A1, 0463706 (GIST-BROCADES N.V.), 2 January 1992 (02.01.92), claims 1-19 --	1,9,10,13
P,X	WO, A1, 9421785 (NOVO NORDISK A/S ET AL.), 29 Sept 1994 (29.09.94), claims 1-17,26 --	1-3,9-10, 13-14
P,X	WO, A1, 9404035 (NOVO NORDISK A/S ET AL.), 3 March 1994 (03.03.94), page 9, line 18 - line 20, claims 1,11 --	1,5-6,8-9, 11-13,16

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

Information on patent family members

03/05/95

International application No.
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		AU-B- 654147	27/10/94
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		JP-T- 5501657	02/04/93
		US-A- 5358864	25/10/94
		WO-A- 9201389	06/02/92
		WO-A- 9201793	06/02/92
WO-A1- 9421785	29/09/94	NONE	
WO-A1- 9404035	03/03/94	NONE	